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A Map of Simian Virus 40 Transcription Sites Expressed in Productively Infected Cells

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The topographical location of "early" and "late" sequences on the physical map of the simian virus 40 genome was determined by reacting unlabeled RNA from monkey cells productively infected with simian virus 40 with the separated strands of each of the 11 DNA fragments formed by digesting supercoiled virus DNA with the restriction endonuclease of *Hemophilus influenzae*. Stable species of early RNA are complementary to the minus strands of the contiguous fragments A, H, I and B, while the late RNA is transcribed predominantly from the plus strands of fragments A, C, D, E, K, F, J, G and B, which also form a continuous set on the physical map. This result is in agreement with previous findings, which indicated that transcription before DNA synthesis is confined to the minus DNA strand, while late transcription includes sequences located predominantly on the plus strand.

The direction of DNA transcription on the plus and minus template strands was ascertained by first preparing a population of linear simian virus 40 DNA molecules oriented by cleavage with the *Escherichia coli* R_1 restriction endonuclease and then digesting them with *E. coli* exonuclease III. DNA reassociation studies using the separated strands of these "half-molecules" and specific simian virus 40 DNA fragments indicated that transcription proceeds from A → H → I → B on the minus DNA strand, and in the opposite direction on the plus DNA strand.

1. Introduction

Transcription of the simian virus 40 genome in productively-infected cells occurs in two recognizable stages. Before the onset of virus DNA synthesis a portion of the genome is transcribed into stable "early" RNA (Aloni *et al.*, 1968; Oda & Dulbecco, 1968; Sauer & Kidwai, 1968; Carp *et al.*, 1969). Following the onset of virus DNA

synthesis, early RNA synthesis continues and is accompanied by the appearance of a new species of RNA; together, these RNA species are complementary to the equivalent of one strand of SV40† DNA (Aloni *et al.*, 1968; Martin & Axelrod, 1969). Recently it has been found that early SV40 RNA is transcribed from 30 to 40% of one strand of the virus DNA (designated the minus strand), while the specifically late SV40 RNA (i.e. RNA found only after the onset of virus DNA synthesis) is transcribed from 60 to 70% of the plus strand (Khoury & Martin, 1972; Lindstrom & Dulbecco, 1972; Khoury *et al.*, 1972; Sambrook *et al.*, 1972). Although these results have clearly identified the early and late template strands and have indicated what fraction of each strand is transcribed into stable virus RNA, they do not indicate the topology of early and late regions of the SV40 genome.

To localize the early and late template regions we have made use of specific fragments of SV40 DNA generated by bacterial restriction endonucleases. Of most use have been the 11 fragments produced by *Hemophilus influenzae* restriction endonuclease (Danna & Nathans, 1971), which vary in size from 22·5% to 4% of the length of the SV40 genome. The order of these fragments in the SV40 DNA molecule is now known (Danna & Nathans, 1972), as is their relation to the cleavage sites of other restriction enzymes, thus providing a physical map of the SV40 genome (Danna *et al.*, 1973). We have used these enzyme cleavage products to determine the regions of the plus and minus strands of SV40 DNA that are transcribed into stable RNA in productively infected cells, and also to determine the direction of early and late transcription. In a subsequent communication, we shall present a similar analysis of SV40 transcription in virus transformed cells.

2. Materials and Methods

(a) *Preparation of virus and virus DNA*

Small plaque SV40 (from strain 776, obtained from K. Takemoto) was plaque purified and a stock prepared in the BSC-1 line of African green monkey kidney cells, as described previously (Danna & Nathans, 1971). ^{32}P-labeled SV40 DNA I was obtained from virus purified by isopycnic centrifugation in CsCl as described previously (Yoshiike, 1968; Trilling & Axelrod, 1970). The specific activities of the labeled DNA preparations varied from 1 to 4×10^5 cts/min per μg. ^{14}C-labeled SV40 DNA I was extracted from infected monkey cells by differential salt precipitation (Hirt, 1967) and purified by equilibrium density centrifugation in CsCl/ethidium bromide (Radloff *et al.*, 1967) followed by sedimentation through a neutral sucrose gradient; the specific activity was 3×10^4 cts/min per μg.

(b) *Digestion of SV40 DNA with* H. influenzae *restriction endonuclease*

To obtain specific fragments of SV40 DNA, [^{32}P] or [^{14}C]DNA I was incubated with *H. influenzae* restriction endonuclease in a mixture containing 25 to 70 μg of DNA, 0·03 unit of enzyme (Smith & Wilcox, 1970), 50 mM-NaCl, and 6 mM each of $MgCl_2$ and Tris·HCl (pH 7·5), in a volume of 0·10 ml, as described previously (Danna & Nathans, 1972). After incubation at 37°C for 2 h, the reaction was complete as determined by electrophoresis and autoradiography of a portion of the reaction mixture (see below).

(c) *Isolation of DNA fragments*

The 11 fragments of SV40 DNA present in an *H. influenzae* enzyme digest (fragments A to K) were separated by electrophoresis in 4% polyacrylamide slab gels measuring 15 cm × 40 cm × 0·16 cm, as described elsewhere (Danna & Nathans, 1972). ^{32}P-labeled

† Abbreviation used: SV40, simian virus 40.

fragments were detected by autoradiography of the wet gel slab and DNA was eluted from segments of gel containing individual fragments with 0·1 times SSC (SSC is 0·15 M-NaCl, 0·015 M-sodium citrate). To localize ^{14}C-labeled fragments, the ^{14}C-labeled digest was mixed with a small amount of ^{32}P-labeled digest and the above procedure followed. The ^{32}P was then allowed to decay to undetectable levels before use of the ^{14}C-labeled fragments. Individual ^{14}C-labeled fragments were then tested for purity by electrophoresis and autoradiography of dried gel slabs; all fragments were pure by this criterion.

(d) *Cleavage of SV40 DNA with* E. coli R_I *restriction endonuclease*

E. coli R_I restriction endonuclease (Yoshimori, 1971) generously supplied by H. Boyer, was incubated with ^{32}P-labeled SV40 DNA I as previously described (Danna *et al.*, 1973). The product had a sedimentation rate of 14·5 S in a 5% to 20% neutral sucrose gradient, corresponding to full length linear molecules (Morrow & Berg, 1972).

(e) *Exonuclease III digestion of* R_I *linear DNA*

The ^{32}P-labeled linear DNA product of the *E. coli* R_I cleavage was incubated at 35°C with 10 units of *E. coli* exonuclease III (kindly provided by H. Gerry) in a reaction mixture containing 5 μg of DNA, 5 mM-$MgCl_2$, 5 mM-β-mercaptoethanol, 50 μg bovine serum albumin/ml, and 50 mM-Tris·HCl (pH 7·4) in a volume of 0·21 ml. Portions of 5 μl of the reaction mixture were removed at various times and the percentage of DNA soluble in cold 5% trichloroacetic acid determined. The values observed were $< 0{\cdot}1\%$, 24%, 45% and 49% at 0, 20, 40 and 60 min, respectively. The reaction was stopped at 60 min by addition of an equal vol. of water-saturated phenol and the aqueous layer plus two 0·2-ml washes of the phenol layer were extracted 5 times with ether. The ether was removed by a stream of nitrogen and the solution was then dialyzed against 0·05 M-NaCl. The exonuclease-treated DNA sedimented in an alkaline sucrose gradient as a broad band with an S value of 13·5 (expected value for half-length strands is 11·5 S), suggesting some variability in the extent of exonuclease digestion of individual molecules. Since exonuclease III digests duplex DNA from the 3′ end, this preparation will be referred to as 5′ "half molecules".

(f) *Preparation of RNA*

"Early lytic" RNA was prepared from BSC-1 cells 24 to 36 h after infection with SV40 (multiplicity of 40 to 80 plaque-forming units/cell) in the presence of 20 μg arabinosyl cytosine/ml as described previously (Khoury *et al.*, 1972). Late lytic RNA was purified from BSC-1 cells 40 to 60 h after infection using the same multiplicity of virus. SV40 complementary RNA was prepared *in vitro* with *E. coli* RNA polymerase and SV40 DNA I as described previously (Khoury & Martin, 1972).

(g) *Separation and purification of SV40 DNA strands*

The ^{32}P-labeled fragments of SV40 DNA, obtained by digestion with the *H. influenzae* restriction endonuclease, were dialyzed against 10 mM-Tris·HCl (pH 7·5), and 10 mM-NaCl. Reaction mixtures containing approximately 0·05 to 0·15 μg of a specific, heat-denatured DNA fragment, 10 μg of complementary RNA, 10 mM-NaCl, 10 mM-Tris·HCl in 1·5 ml were incubated at 60° for 1 h. The plus and minus strands of each of the 11 fragments were then separated on hydroxyapatite and were purified by methods described previously (Khoury *et al.*, 1972).

(h) *DNA-RNA hybridization*

Small amounts (1 to 5 ng) of the plus or minus strand of each specific SV40 DNA fragment were incubated with early (0·2 to 0·5 mg) or late (0·1 to 0·3 mg) lytic RNA in 1·0 M-NaCl, 80 mM-phosphate buffer and 0·2% sodium dodecyl sulfate in 0·15 to 0·25 ml at 68°C for 18 to 24 h. DNA–RNA hybrid formation was monitored on hydroxyapatite (Kohne, 1969). DNA–RNA hybrids formed with fragments A or B, and lytic RNA, were also examined by treatment with the single-strand specific nuclease, S_1, as has been described in a previous publication (Khoury *et al.*, 1973). The reaction mixtures for S_1 nuclease

analysis were diluted to 3 ml to give a final concentration of 300 mM-NaCl, 10^{-2} mM-$ZnSO_4$, 30 mM-sodium acetate buffer (pH 4·0) and 30 μg denatured salmon sperm DNA/ml, and divided into 3 equal portions, each containing about 350 cts/min of ^{32}P-labeled DNA. 2 of the samples were treated with an excess of the S_1 enzyme and the third served as a control. The fraction of the DNA resistant to the single strand specific enzyme was determined from the average of the nuclease resistant radioactivity in the 2 treated samples compared to the untreated sample.

(i) *DNA–DNA hybridization*

DNA–DNA hybridization was carried out with ^{14}C-labeled denatured fragments of SV40 DNA and the separated strands of the ^{32}P-labeled 5′ half-molecules produced by sequential cleavage of DNA I with *E. coli* R_I endonuclease and exonuclease III. The strands of 5′ half molecules were separated as described above for the separation of the strands of DNA fragments. These separated strands will be referred to as the plus and minus 5′ half strands. For hybridization, approximately 1 to 4 ng of either the plus or minus 5′ half strand was incubated with approximately 20 to 40 ng of a specific heat-denatured ^{14}C-labeled DNA fragment in 1·2 M-NaCl, 100 mM-phosphate buffer, 0·3% sodium dodecyl sulfate for 24 h at 68°C. The extent of DNA reassociation was then analyzed on hydroxyapatite (Britten & Kohne, 1968).

(j) *Detection of radioactivity*

After the addition of 0·2 mg of carrier yeast RNA, samples were adjusted to 5% trichloroacetic acid and collected on nitrocellulose membrane filters (B-6, Carl Schleicher and Schuell Co., Keene, N. H.). The filters were washed with 10 ml of cold 0·01 N-HCl, dried, and counted in a toluene-based scintillation fluid. A background of 20 cts/min was subtracted from each result.

3. Results

(a) *Separation of fragment strands*

The plus and minus strands of each of the 11 fragments (A to K), produced by digestion of SV40 DNA I with the *H. influenzae* restriction endonuclease, were separated by incubating denatured fragments with an excess of SV40 complementary RNA followed by chromatography on hydroxyapatite, as described previously for unfractionated virus DNA (Khoury *et al.*, 1972). Less than 4% of the purified separated strands of each fragment "self-associated" when incubated under the conditions used for DNA–RNA hybridization. The strand which reacted with SV40 complementary RNA has been designated the minus strand, and the other, the plus strand.

(b) *Hybridization of the plus and minus strands of DNA fragments with RNA from SV40-infected BSC-1 cells*

As shown previously, 30 to 40% of the minus strand of SV40 DNA is expressed early in a productive infection and 60 to 70% of the plus strand is expressed late in infection (Lindstrom & Dulbecco, 1972; Khoury *et al.*, 1972; Sambrook *et al.*, 1972). In order to determine which of the 11 DNA fragments contained the sequences complementary to early RNA and to late RNA, the individual strands of each DNA fragment were incubated with early or late lytic RNA. The percentage of each [^{32}P] DNA fragment which hybridized to added RNA was then determined by hydroxyapatite chromatography. The results of these experiments, shown in Table 1, indicated a complex pattern of transcription.

TABLE 1

Hybridization of early or late lytic RNA to the separated strands of unique SV40 DNA fragments

DNA fragment	[^{32}P]DNA in hybrid molecules (%)			
	(−) DNA strand		(+) DNA strand	
	Early RNA	Late RNA	Early RNA	Late RNA
A	87	91	9	83
B	87	85	12	87
C	9	46	15	90
D	5	14	19	88
E	8	12	16	90
F	3	7	21	91
G	10	14	16	91
H	39	51	6	35
I	23	30	7	46
J	10	10	14	85
K	5	7	12	87

RNA from SV40-infected BSC-1 cells (early, 0·2 to 0·5 mg; late, 0·1 to 0·3 mg) was hybridized under the conditions described in Materials and Methods to the plus or minus strand of each SV40 DNA fragment produced by cleavage with the *H. influenzae* restriction endonuclease. Samples containing 250 to 400 cts/min were analyzed by hydroxyapatite chromatography to determine the percentage of [^{32}P]DNA in hybrid molecules. In the absence of RNA, this value was less than 4% for either strand of the 11 fragments. Similarly, low levels of hybridization (< 2%) were found when uninfected monkey cell RNA was incubated with either strand of a control fragment (E).

Focusing first on the reaction between early RNA and the minus strands of DNA fragments, which are derived from the early template strand (column 1 of Table 1) we see that only fragments A, B, H and I hybridized with early RNA to a significant extent. At the level of RNA used, fragments A and B were essentially saturated, whereas H and I were not. Therefore, we conclude that at least a portion of the SV40 genome corresponding to each of the A, B, H and I fragments is transcribed into early SV40 RNA, and that stable "early" transcripts from segments A and B are probably more abundant than those from H and I.

Considering next the reaction between late RNA and the plus strands of the DNA fragments, which are derived from the specifically late template strand (column 4 of Table 1), we see that all fragments hybridized with late RNA. At the level of RNA used, fragments A, B, C, D, E, F, G, J and K were essentially saturated, whereas fragments H and I were not. We conclude from these results that at least a portion of the SV40 genome corresponding to each of the fragments is transcribed into late RNA and that stable late transcripts from A, B, C, D, E, F, G, J and K are probably more abundant than those from H and I.

The reaction between the minus strands of DNA fragments and late RNA (column 2 of Table 1) shows the expected hybridization with fragments A, B, H and I since early SV40 RNA species are known to be present during the late phase of productive infection (Aloni *et al.*, 1968; Oda & Dulbecco, 1968; Sauer & Kidwai, 1968; Carp *et al.*, 1969). In addition, the minus strand of fragment C is unique in that it hybridizes with late SV40 RNA but not with early RNA. The possible significance of this last result will be considered in the Discussion.

Also shown in Table 1 (column 3) is the reaction between early RNA and the plus strands of DNA fragments. As shown in the Table, the plus strands of several fragments showed a low level of hybridization with early RNA. The significance of the low levels of reaction between plus (late template) strands of some of the other fragments and early RNA is not clear (see Discussion).

(c) *Hybridization of fragments H and I with different concentrations of RNA*

The minus strands of fragments A and B and the plus strands of all fragments except H and I were saturated by the amounts of late lytic RNA used in this study. The low levels of hybridization of both the plus and minus strands of fragments H and I with late RNA (Table 1) suggested that stable transcripts from these regions of SV40 DNA may be less abundant than transcripts from other regions of the genome. To explore this possibility further, the hybridization reaction with these fragments was done with different concentrations of late RNA. As shown in Table 2, the extent

TABLE 2

Effect of RNA concentration on hybridization experiments with DNA fragments H and I

DNA fragment	Strand	RNA concentration† (mg/ml)	Duplex molecules (%)
H	+	2·4	16
		3·2	35
H	−	2·4	39
		3·2	51
I	+	2·4	27
		3·2	46
I	−	2·4	24
		3·2	30
J	+	1·6	85
J	−	1·6	10
K	+	1·2	87
K	−	1·2	7

Under conditions described in Materials and Methods, 1 to 4 ng (approx. 300 cts/min) of either strand of a DNA fragment was incubated with the indicated concentrations of late lytic RNA. The percentage of radioactivity in duplex molecules was analyzed by hydroxyapatite chromatography.

† Total cellular RNA obtained late in the productive cycle of SV40 infection of BSC-1 cells.

of hybridization of each strand of fragment H and of fragment I was dependent on the concentration of late RNA. This was the case even at RNA levels which were at least two to three times higher than that needed to saturate the plus strand of the other DNA fragments, including fragments J and K which are smaller than H and I. These data, together with those presented in Table 1, thus suggest that stable transcripts of both strands of fragments H and I are present in lower relative concentrations than RNA species transcribed from SV40 DNA sequences corresponding to the other nine DNA fragments. Alternatively, the results obtained with fragments H and I could point to a previously unsuspected heterogeneity of these fragments (see Discussion).

(d) *Estimation of the proportion of each strand of fragments A and B which hybridize with lytic RNA*

The results presented in Table 1 indicate that more than 85% of the plus and minus strands of fragments A and B reacted with late lytic RNA. In this experiment, the DNA–RNA reaction was monitored by hydroxyapatite chromatography which fails to discriminate between complete and partial DNA–RNA hybrids. For this reason we decided to use the S_1 single-strand specific nuclease assay to determine the fraction of the minus and plus strands which form DNA–RNA hybrids. Since early SV40 RNA sequences are represented in late lytic RNA, and since there is more SV40-specific RNA late after infection, late lytic RNA was used in these studies to insure that the DNA–RNA reactions had reached saturation.

The separated strands of ^{32}P-labeled fragments A and B were incubated with saturating amounts of late lytic RNA and then exposed to the S_1 nuclease as described in Materials and Methods. The proportion of either DNA strand of these fragments which formed hybrid structures with RNA was determined as the percentage of [^{32}P]DNA resistant to the S_1 enzyme. This value provided an estimate of the extent of transcription on each strand of fragment A or B (Table 3). Approximately 33% of the plus strand and 60% of the minus strand of fragment A appear to be transcribed, while 51% of the plus strand and 40% of the minus strand of fragment B are expressed. Since each of these sets adds up to nearly 100%, one interpretation of these results is that the minus and plus strand templates do not overlap.

TABLE 3

Extent of hybridization of lytic RNA to a specific DNA fragment

DNA fragment	Strand	Resistance of hybrid to S_1 enzyme (%)
A	+	33
A	−	60
B	+	51
B	−	40

Approximately 5 ng of the plus or minus strand of fragments A and B were incubated with 0·8 to 1·0 mg of late lytic RNA in 0·75 ml as described in Materials and Methods. After 24 h incubation, the sample was diluted to 3 ml and divided into thirds; 2 of these were treated with an excess of S_1 enzyme, while the third served as a control. The percentage resistance to S_1 nuclease is the average of the radioactive counts in the 2 treated samples compared to the control. The values in this Table represent the average of 2 of these experiments and differed by less than 10%

(e) *Map positions of early and late template regions*

Danna *et al.* (1973) have constructed a physical map of the SV40 genome based on the sites of cleavage of SV40 DNA by restriction endonuclease from *H. influenzae*, *H. parainfluenzae*, and *E. coli* (R_T) (Fig. 1). We can therefore relate the results of hybridization of DNA fragments with SV40 lytic RNA to the map position of individual fragments.

Early RNA hybridized to the minus strands of fragments A, H, I and B, which form a contiguous group in the map (Fig. 1). Since only 60% of the minus strand of A and

40% of the minus strand of B are expressed, the early template region appears to extend from near the middle of A to near the middle of B, i.e. about 30% of the length of the virus genome. This interpretation assumes that the DNA sequences transcribed into stable early RNA are entirely contiguous. Late lytic RNA, on the other hand, hybridized almost completely with the plus strands of fragments A, C, D, E, K, F, J, G and B. As seen in the cleavage map shown in Figure 1, these fragments also form a contiguous group. Since about 33% of the plus strand of A and about 51% of the plus strand of B are expressed, the late template region forms a segment encompassing about 70% of the length of the virus genome (Fig. 1). As noted above, very high concentrations of late lytic RNA hybridize with the plus strands of fragments H and I but the reaction is incomplete. This suggests that a minor species of late lytic RNA is transcribed from parts of the sequences corresponding to fragments H and I (see Discussion).

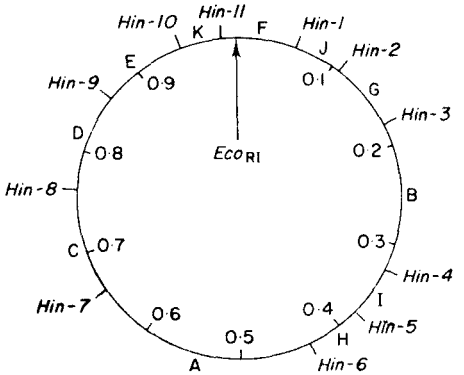

FIG. 1. A cleavage map of the SV40 genome (Danna *et al.*, 1973). The *E. coli* R_I endonuclease site (*Eco* R_I) is the zero point and map distances are given as fractional length of SV40 DNA in the direction F → J → G– – –. *Hin-1*, *2*, *3*, etc. refer to the *H. influenzae* endonuclease cleavage sites. The map positions of these are: *Hin-1*, 0·060; *Hin-2*, 0·105; *Hin-3*, 0·175; *Hin-4*, 0·325; *Hin-5*, 0·375; *Hin-6*, 0·430; *Hin-7*, 0·655; *Hin-8*, 0·760; *Hin-9*, 0·860; *Hin-10*, 0·945; *Hin-11*, 0·985.

(f) *Direction of transcription*

Although the results just presented indicate the position of early and late template regions on the SV40 map, they do not indicate the direction of transcription of the early and late genes, i.e. clockwise or counter-clockwise transcription as the map is drawn. To determine the direction of transcription, it is sufficient to establish the 5′ to 3′ direction of the minus and plus strands of SV40 DNA relative to the cleavage map, since RNA is transcribed in the 3′ to 5′ direction of the template strand, and the minus and plus strands are known to be templates for early and late RNA, respectively.

In order to relate the 5′ to 3′ direction of the minus and plus strands of SV40 DNA to the cleavage map, we have carried out the experiment diagrammed in Figure 2. ^{32}P-labeled SV40 DNA I was first cleaved with the *E. coli* R_I restriction endonuclease to obtain unique, full length linear molecules. Since the R_I enzyme cleaves within fragment F (Danna *et al.*, 1973), these molecules have the map order F_1 J G B I H A

C D E K F_2 (see Fig. 2). In the next step, the linear molecules were digested with *E. coli* exonuclease III, which removed the 3′ halves of each strand, leaving 5′ half molecules. The individual minus and plus 5′ half strands were then isolated by annealing with SV40 complementary RNA. Finally, we determined which 5′ half strand (plus or minus) contains fragment J and fragment G sequences, and which contains fragment K and fragment E sequences by annealing each half strand with denatured ^{14}C-labeled fragments J, G, K or E. The sequences corresponding to these four fragments are nearest to sequence F, which is present at each end of the R_I linear molecules (Fig. 2).

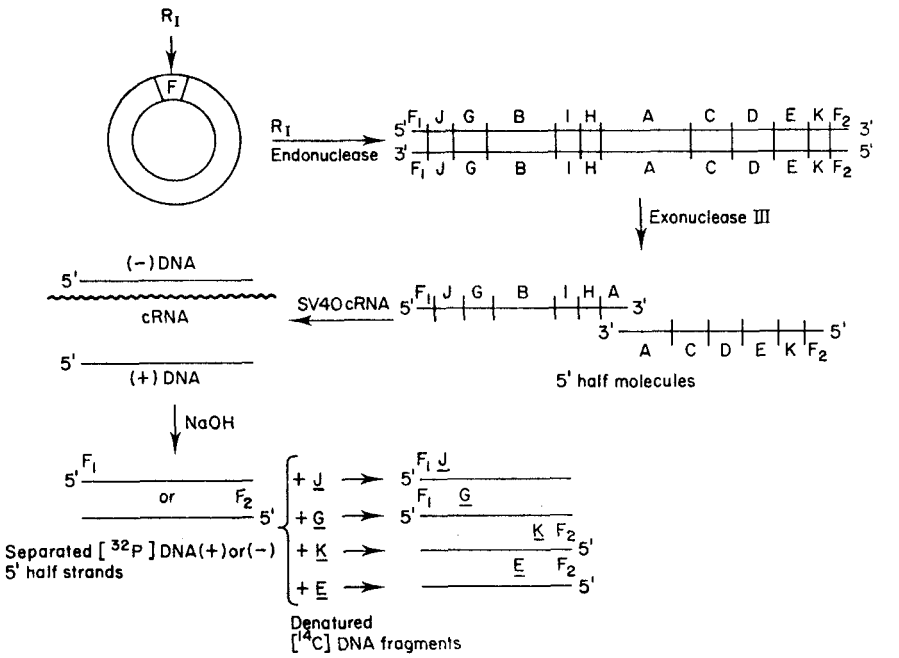

FIG. 2. Scheme for determining the 5′→3′ orientation of SV40 DNA strands. See the text for a description of each step. F_1 and F_2 are the parts of fragment F resulting from cleavage by the R_1 restriction enzyme. cRNA, complementary RNA.

The results of duplicate experiments are presented in Table 4. Plus or minus ^{32}P-labeled 5′ half strands were incubated with a tenfold excess of a single, denatured, ^{14}C-labeled DNA fragment (G, J, E or K) under annealing conditions, and the fraction of [^{32}P]DNA which formed duplexes with the [^{14}C]DNA was determined by hydroxyapatite chromatography. The results indicate that fragments E and K hybridize preferentially with the *plus* 5′ half strand, while fragments G and J hybridize preferentially with the *minus* 5′ half strand of SV40 DNA. In each case, more than 85% of the ^{14}C-labeled DNA fragments reassociated. Theoretically one would expect that nearly all of the ^{32}P-labeled DNA would anneal in the presence of an excess of the appropriate [^{14}C]DNA fragment if the half-strands had remained intact. However, when the separated ^{32}P-labeled 5′ half strands used in this experiment were examined in alkaline sucrose, they sedimented in the 5·4 to 10·7 S region of the gradient which corresponds to a single-stranded molecule 1·7 to $6{\cdot}1 \times 10^5$ daltons in size, thus explaining why no more than 40% of the [^{32}P]DNA reassociated. The data, nevertheless, are

TABLE 4

Hybridization of the separated strands of ^{32}P-labeled SV40 DNA half-molecules with specific ^{14}C-labeled SV40 DNA fragments

Denatured [^{14}C]DNA fragment	[^{32}P]DNA in hybrid molecules (%)	
	(+) DNA strand	(−) DNA strand
G	6	40
J	11	35
E	40	20
K	30	15

Hybridization reactions were done as described in Materials and Methods. The percentage of [^{32}P]DNA in hybrid molecules was analyzed on hydroxyapatite as previously described. Values represent the average of 2 experiments and differed by less than 10%.

internally consistent, i.e. adjacent fragments E and K preferentially bind to one 5′ half strand (plus), while fragments G and J bind preferentially to the other (minus) 5′ half strand. Since the minus strand is the template for early SV40 RNA, we can conclude that the orientation of the early template strand is 5′ F J G B I H A . . . . . 3′, i.e. 5′—3′ clockwise on the cleavage map shown in Figure 1, and the orientation of the plus strand (late template strand) is 5′ F K E D C A . . . . 3′, i.e. 5′—3′ counterclockwise on the cleavage map. It follows, therefore, that transcription of early genes proceeds from A to B counter-clockwise on the minus DNA strand and transcription of late genes proceeds from A to B in a clockwise direction on the plus DNA strand.

4. Discussion

The major conclusions of this study are presented in the map shown in Figure 3. We have confirmed previous results indicating that early in productive infection, transcription of the SV40 genome occurs on the minus strand template. Later in infection, additional transcription occurs on the plus strand template. The early region of SV40 DNA includes segments of fragments A, B, H and I, while the late region of the virus genome encompasses polynucleotide sequences present in fragments C, D, E, K, F, J, G and parts of A and B. In addition, there is evidence for late transcription of at least part of the sequences corresponding to the plus strands of fragments H and I. These transcripts, however, are at a much reduced level compared to those from the other parts of the genome. Within fragments A and B, which have relatively abundant RNA species complementary to each strand, nearly all of the stable virus-specific RNA appears to be asymmetrically transcribed, since in each case we were able to account for over 90% of the transcribing activity by summing the hybridization to each DNA strand. Although we cannot make any statement about the precise location of transcribed regions on the plus and minus strands of fragments A and B, the simplest explanation of this result is that the parts of A and B nearest H and I are transcribed from the minus strand template and the parts nearest C and G, respectively, are transcribed from the plus strand template. The early region would then encompass sequences present between 0·26 to 0·57 map unit and the late region would comprise the rest of the genome (Fig. 3).

The direction of transcription of early and late SV40 RNA has been established by

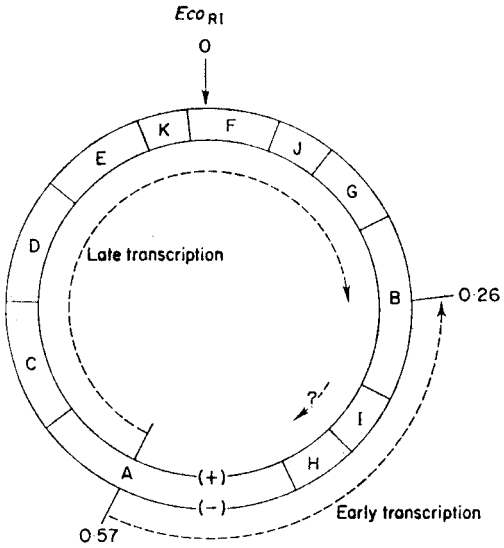

FIG. 3. Diagrammatic representation of transcription, early and late in the SV40 lytic cycle as it relates to the physical map of the viral genome.

determining the 5′ to 3′ orientation of the minus and plus strands of SV40 DNA, which serve as the early and late templates, respectively. As indicated in Figure 3, early transcription occurs in a counter-clockwise direction from A to B and late transcription occurs in a clockwise direction from A to B. On the assumption that the abundant species of stable SV40 RNA that we have used in these studies is the immediate transcription product, we can localize initiation sites for both early and late transcription near the middle of fragment A (at 0·57 map unit) and termination sites for both early and late transcription near the middle of fragment B (at 0·26 map unit). Alternatively, if large regions of the DNA are symmetrically transcribed (Aloni, 1972) with subsequent degradation of specific RNA sequences, the true initiation and termination sites may be at different locations. Furthermore, we have not excluded the possibility that other initiation and termination sites may be present within both the early and late regions. In this regard, experiments carried out *in vitro* with *E. coli* RNA polymerase and an SV40 DNA I template suggest that transcription with this enzyme begins outside fragment A (Westphal *et al.*, 1973; Zain, Dhar, Weissman, Lebowitz & Lewis, unpublished observations). It will be of considerable interest to determine what special nucleotide sequences may exist at the transition sites within fragments A and B, and whether these sites are related to the specific cleavage of SV40 component I DNA by the single-strand specific nuclease S_1 (J. Morrow & P. Berg, personal communication).

The map position for the early region of the SV40 genome shown in Figure 3 is entirely consistent with observations made with adeno-SV40 hybrid viruses by Kelly & Lewis (personal communication) who found that the entire early region of SV40 is included in the DNA of a hybrid virus containing 43% of the SV40 genome. They have localized within the early segment those regions which are required for expression of U antigen, T antigen, and the SV40 tumor-specific transplantation antigen. Recently

Patch *et al.* (1972) found that the SV40 DNA segment of $AD2^{+}ND_1$ contains parts of both early and late information and, therefore, has a probable transcriptional initiation or termination site. A comparison of the map of the SV40 segment of the adenovirus hybrid with the cleavage map of the SV40 genome (Morrow & Berg, 1972; T. Kelly, personal communication; P. Lebowitz, personal communication) suggests that this site corresponds to the termination point within fragment B. Therefore, it is likely that transcription of SV40 DNA sequences in $AD2^{+}ND_1$ infected cells begins within the adenovirus portion of the DNA.

The incomplete hybridization of *both* the minus and plus strands of fragments H and I with the RNA from infected monkey cells (Table 1) suggests that transcription of SV40 DNA may be more complex than initially thought. As pointed out above, fragments H and I, which are physically located in the middle of the early region of the viral genome, were the only two that failed to react completely with amounts of lytic RNA known to be saturating for the other nine *H. influenzae* DNA fragments. Although heterogeneity of these two fragments could explain this finding, there is at present no other evidence for heterogeneity (Danna *et al.*, 1973). The results presented in Table 2 suggest that the relative abundance of RNA complementary to fragment H or I DNA is much lower than the other, stable lytic RNA species encountered thus far. It is not clear at present whether the low concentrations of virus specific RNA complementary to DNA fragments H and I reflect a diminished rate of synthesis or the degradation of specific polyribonucleotide sequences transcribed from this region of the virus genome. Either of these possibilities would reduce the concentrations of specific RNA sequences and explain the results presented in Table 2. The reaction of a significant proportion of the *plus* strands of fragments H and I with late lytic RNA (Tables 1 and 2) suggests the existence of late DNA sequences (or early information on the plus DNA strand) within the early segment of the virus DNA. As such, this class of RNA may have an important role with respect to the regulation of SV40 DNA transcription.

Low levels of reaction (6 to 21%) were also observed between early lytic RNA and the plus strands of the 11 SV40 DNA fragments (Table 1). Previous reports have indicated that early RNA is complementary to the minus strand of virus DNA (Lindstrom & Dulbecco, 1972; Khoury *et al.*, 1972; Sambrook *et al.*, 1972). The early lytic RNA used in the experiment shown in Table 1 was prepared from monkey cells infected in the presence of arabinosyl cytosine. Incomplete inhibition of SV40 DNA synthesis at a time during productive infection (24 to 36 h) when late RNA is known to be present may account for the observed low levels of reaction between early RNA and the plus strands of the various fragments. Alternatively, this result could reflect the incomplete post-transcriptional degradation of RNA complementary to the plus strand of SV40 DNA. Several reports have appeared recently which suggest that integration of virus genetic information into chromosomal DNA may occur during productive infection by SV40 (Lavi & Winocour, 1972; Tai *et al.*, 1972; Hirai & Defendi, 1972). Whether the RNA transcribed from such integrated SV40 DNA (Jaenisch, 1972; Rozenblatt & Winocour, 1972) is related to those RNA sequences that hybridize incompletely with the DNA fragments remains to be determined.

The partial hybridization of late, but not early RNA, to the minus strand of fragment C (Table 1) is worthy of comment. Since fragment C contains the origin of DNA replication (Nathans & Danna, 1972; Danna & Nathans, 1972), it is possible that this RNA represents a primer for initiation of DNA replication (Wickner *et al.*, 1972). Since SV40 DNA replication proceeds bidirectionally from fragment C (Danna &

Nathans, 1972; Fareed *et al.*, 1972), one might expect primers for each strand at the initiation site.

It has been shown that the pattern of transcription in SV40 transformed cells is significantly different from that observed during lytic infection (Khoury *et al.*, 1973). The use of SV40 DNA fragments produced by restriction endonucleases should permit a topographic analysis of these differences.

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